VOLUME XVII OCTOBER, 1927 PART IV

BIOCHEMISTRY OF WATER-LOGGED SOILS. PART I.

THE EFFECT OF WATER-LOGGING ON THE DIFFERENT FORMS OF NITROGEN, ON THE REACTION, ON THE GASEOUS RELATIONSHIPS, AND ON THE BACTERIAL FLORA[1].

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(With Eight Graphs.)

WATER-LOGGING of soils is a common phenomenon, especially in the tropics where the rains are heavy and rivers frequently overflow their banks or lakes burst their bounds; vast areas often remain for long periods submerged. The rice fields—which cover an area of about eighty million acres in India alone—have to be maintained for months in the swamp state. The chemical and biological changes which attend such conditions are therefore of great economic importance and considerable scientific interest.

The scientific literature on the subject is comparatively limited. In 1881 Warington (26) carried out some laboratory trials and found that he could not completely recover added nitrates when the soil remained covered with water. Nagaoka (17) observed that nitrites were formed as a result of heavy dressings of nitrates to rice fields. Daikuhara and Imasaki (4) noted considerable reduction in the soluble nitrogen. Kelly (12) observed that swamping led to considerable fall in nitrate content and that the nitrites formed were toxic to plants. Oelsner (19) stated that increased moisture content of soils beyond 20 per cent. led to inevitable loss of nitrates and that at 40–50 per cent. even larger quantities of total nitrogen were lost to the soil.

Harrison and Iyer (11) carried out researches on the gases of swamp rice soils and found that methane, hydrogen, carbon dioxide and nitrogen were the chief gases present in the soil, but that by the combined action of certain aerobic bacteria, green algae and the roots of plants, they

[1] Parts I to IV of this series were submitted to the Royal Agricultural Society of England in 1926 as a Thesis, for which the author was awarded the Research Medal of that Society.

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were oxidised and decarbonised, resulting in the evolution of only free oxygen and nitrogen.

It is desirable to have detailed knowledge of the more fundamental changes and the object of the work described in this series of papers is to obtain quantitative data regarding the effect of water-logging on the various forms of nitrogen, the oxygen, carbon dioxide and acidity relations, the bacterial numbers and other attendant biological phenomena.

Experimental.

The soils taken for detailed investigation were (1) Soil from Plot 1,0 of Barnfield, Rothamsted, which has been under root-cultivation since 1856 and receives 14 tons of dung to the acre annually; (2) Garden soil from Central Farm, Coimbatore, South India.

The samples were air-dried, freed from undecomposed plant residues, passed through a 1 mm. sieve, shaken well with water (100 parts of soil in 250 parts of water) and incubated for 40 days, the Rothamsted soil at 20° C. and the Indian soil at 35° C. Samples were taken at three-day intervals for analysis.

The Effect of Water-logging on the Nitrogen Compounds.

Gaseous ammonia evolved from the soils was estimated by absorption in 0·004*N* acid and back-titration against alkali of the same strength.

Very minute but definite quantities (1·6 P.P.M. from the Rothamsted soil and 1·2 P.P.M. from the Indian soil) were evolved within the first 24 hours, but after that time the amounts evolved were not appreciable (Graph 1).

Free and saline ammonia were estimated by an aeration method (20).

A very distinct increase was noticeable even at the end of the first 24 hours. The rise continued up to the end of the seventh day, after which the variations were all within the limits of experimental error (Graph 1).

The rate of formation of ammonia in the Rothamsted soil was about five times that in the Indian soil, being 39 P.P.M. in seven days in the former against only 8 P.P.M. in the latter. In spite of the difference in temperature, ammonia-formation proceeded vigorously in both the soils during the same period of time and then came practically to a stop.

The mechanism of the production of ammonia cannot be purely chemical: amides exist in the soil only in small quantities and are not decomposed even by a 4 per cent. solution of sodium carbonate (20).

Nitrites were estimated by Davisson's titration method (5).

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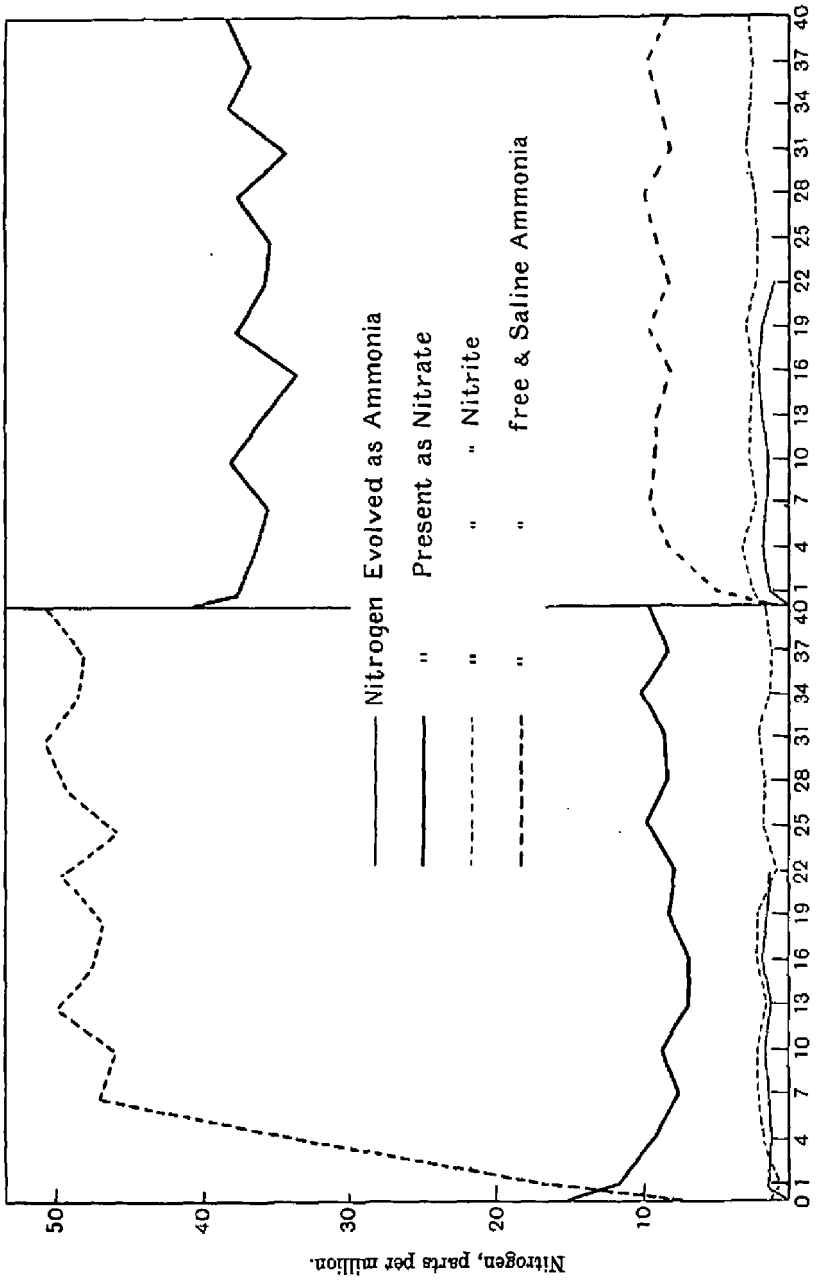

Graph 1. Changes in the different forms of Nitrogen in water-logged soils.

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The two soils showed only very slight increases in the early stages (1·1 P.P.M. at the end of seven days for the Rothamsted soil and 1·4 P.P.M. at the end of four days for the Indian soil), but later a succession of rises and falls was noted, most of which lie within the range of experimental error (Graph 1). A critical examination of the figures shows that although the general average appears to be increased as the result of the water-logging there is not sufficient evidence to conclude that the rise in nitrite-content is real.

Nitrates together with nitrites were estimated by the Devarda's Alloy method (24). The nitrites having been determined separately, the nitrates were obtained by difference. Both the soils showed marked reduction in nitrates (7·1 P.P.M. in the Rothamsted soil and 5·7 P.P.M. in the Indian soil) during the first seven days (Graph 1). The quantities present later seemed to vary only slightly.

It is possible that a part of the nitrate lost was converted into nitrite. A portion may have been reduced to ammonia; but the latter is produced in such large quantities that it could not have come from the nitrates alone. Some nitrates may have been assimilated by micro-organisms or reduced to nitrogen gas.

Total nitrogen was determined by the Kjeldahl method.

As has already been pointed out (21) the relatively high standard error detracts much from the value of the determinations: but these estimations were carried out in view of the fact that, by adopting a similar method, cases of marked loss of nitrogen have been recorded (19).

A study of the figures showed that they did not vary significantly. The average content of total nitrogen was 2439 P.P.M. for Rothamsted soil and 888·5 P.P.M. for Indian soil. The values for different times of sampling varied irregularly about these means, and in no case did the deviation exceed twice the standard error of difference[1] which was ± 27·2 P.P.M. for Rothamsted soil and ± 23·0 P.P.M. for Indian soil. There is thus no evidence to suggest that as a result of water-logging the total nitrogen of the soils suffered any change in amount.

Comparative Study of Nitrogen Changes in various Indian Soils.

It has so far been noted that as a result of the water-logging there were (*a*) a distinct increase in free and saline ammonia, (*b*) significant though slight reduction in nitric nitrogen and (*c*) no diminution at all in total nitrogen. In order to see if these phenomena were general and could be observed on soils which become or are apt to become water-

[1] $\sqrt{2}$ × Standard Error.

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logged frequently, representative specimens from the various provinces of the Indian Empire[1] were obtained and studied for the changes in the three forms of nitrogen.

Free and saline ammonia were estimated in this series by the McLean and Robinson method (15).

A statistical study (7) of the results (see Table I) shows that in spite of the great diversity in origin and character of the soils the increase in free and saline ammonia content was quite pronounced in all cases, even at the end of the third day. The production continued at more than double the rate up to the end of the tenth day.

The formation of ammonia went on much more vigorously in certain soils than in others. There is no correlation whatever between the rate of production and the total nitrogen content or the amount of ammonia originally present.

Table I. *Table showing increased Production of Free and Saline Ammonia in different Indian Soils as a result of Water-logging.*

			Nitrogen as parts per million Free and Saline Ammonia			
No.	Origin of sample	Total Nitrogen	At start	3rd day	7th day	10th day
I.	Dera Ghazi Khan, Punjab	679·7	13·4	21·5	30·6	39·8
II.	Jadiala Baghwala, Punjab	345·3	19·6	15·0	21·3	29·7
III.	Chicoki Malian, Punjab	336·9	10·0	21·7	35·8	46·1
IV.	Chuhur Khana, Punjab	863·3	13·8	24·2	37·3	46·8
V.	Karimganj, Assam	772·4	15·5	16·3	20·3	27·5
VI.	Pausra, Punjab	643·5	11·2	18·9	26·4	36·9
VII.	Marh Bhangwan, Punjab	282·9	10·7	19·4	27·9	35·3
VIII.	Chicharianwali, Punjab	334·4	21·5	22·2	26·9	32·1
IX.	Titabar, Assam	2748·7	7·4	13·4	20·2	25·8
X.	Kaliganj, Bengal	1100·9	13·9	22·0	28·9	36·2
XI.	Dacca, Bengal	1233·1	21·8	26·4	32·0	39·9
XII.	Yessgaon No. 1, Bombay	425·6	9·3	8·8	12·9	16·6
XIII.	Yessgaon No. 2, Bombay	404·2	9·5	9·9	13·4	16·9
XIV.	Kopergaon, Bombay	575·8	9·4	16·5	21·8	26·8
XV.	Malad No. 1, Bombay	711·5	4·9	16·7	23·3	30·2
XVI.	Malad No 2, Bombay	744·9	12·4	12·7	16·9	19·3
XVII.	Karjat, Bombay	570·6	6·7	12·7	20·1	25·2
XVIII.	Hmawbi, Burma	766·8	12·4	12·4	15·4	17·8
XIX.	Mandalay, Burma	545·1	19·0	23·0	30·5	36·3
XX.	Anakapalli, Madras	866·2	59·2	62·3	67·8	71·3
XXI.	Coimbatore F Block	1460·2	27·7	52·3	65·6	76·9
XXII.	Coimbatore M Block	630·8	23·0	29·3	36·1	44·8
XXIII.	Tirur, Madras	2556·6	34·1	40·5	45·7	51·3
XXIV.	Sholavandan, Madras	522·0	27·2	28·7	32·1	34·8

Average increase ($\bar{x}$)÷Standard Error$=t$. $t=2$ is taken as significant.
Values of t: 0–3rd day$=4·7$; 3–7th day$=11·1$; 7–10th day$=11·0$.

[1] The author takes this opportunity to thank the Directors of Agriculture of the various provinces for their willing response to his request for samples.

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Nitrates. A study of the data (Table II) shows that whilst the reduction in the nitrate content on the third day was not marked, that at the end of the seventh day was significant. There was practically no difference between the quantities present on the seventh and the tenth days.

The amounts lost on water-logging do not seem to bear any relation to either the respective total-nitrogen contents or to the quantities of nitrates originally present.

Table II. *Variations in the Nitrate-Content of Different Indian Soils when Water-logged.*

No.	Origin of sample	Total Nitrogen	Nitrogen as parts per million Nitric Nitrogen: At start	3rd day	7th day	10th day
I.	Dera Ghazi Khan	679·7	20·8	19·8	20·2	19·4
II.	Jadiala Baghwala	345·3	5·7	1·5	2·1	1·9
III.	Chicoki Malian	336·9	44·9	35·6	36·8	36·2
IV.	Chuhur Khana	863·3	239·5	247·5	240·2	232·3
V.	Karimganj	772·4	7·9	8·2	7·6	8·0
VI.	Pausra	643·5	13·7	12·6	13·0	12·8
VII.	Marh Bhangwan	282·9	34·3	34·6	33·8	34·2
VIII.	Chicharianwali	334·4	9·9	9·3	8·8	9·2
IX.	Titabar	2748·7	1·7	4·5	3·3	2·9
X.	Kaliganj	1100·9	9·9	12·1	10·9	11·2
XI.	Dacca	1233·1	5·1	2·9	3·6	3·2
XII.	Yessgaon No. 1	425·6	1·4	1·1	1·0	1·2
XIII.	Yessgaon No. 2	404·2	1·6	1·2	1·4	1·0
XIV.	Kopergaon	575·8	14·4	12·8	10·2	9·6
XV.	Malad No. 1	711·5	8·2	7·1	7·3	6·8
XVI.	Malad No. 2	744·9	8·4	8·0	7·6	7·5
XVII.	Karjat	570·6	3·0	2·0	1·3	1·1
XVIII.	Hmawbi	766·8	2·3	3·0	2·7	2·1
XIX.	Mandalay	545·1	5·3	2·3	2·0	2·7
XX.	Anakapalli	866·2	6·0	4·6	4·8	4·4
XXI.	Coimbatore F Block	1460·2	4·2	3·1	3·3	3·8
XXII.	Coimbatore M Block	630·8	12·2	11·1	10·5	10·3
XXIII.	Tirur	2556·6	14·2	8·6	7·9	8·2
XXIV.	Sholavandan	522·0	7·3	6·2	5·7	5·4

Average diminution ($\bar{x}$) ÷ Standard Error = t. $t = 2$ is taken as significant.
Values of t: 0–3rd day, 1·4; 3–7th day = 1·8; 7–10th day = 1·3;
0–7th day = 3·3; 0–10th day = 4·7.

Total nitrogen. An analysis of the results (Table III) shows that in spite of the marked differences between the individual data the average variances are insignificant. There is no evidence to show that any loss in total nitrogen followed as a result of mere water-logging.

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Table III. *Table showing the total Nitrogen Contents at different times in Water-logged Soils.*

No.	Origin of the soil	Total Nitrogen as parts per million		
		At start	15th day	30th day
I.	Dera Ghazi Khan	679·7	663·6	670·3
II.	Jadiala Baghwala	345·3	359·8	340·1
III.	Chicoki Malian	336·9	325·7	331·2
IV.	Chuhur Khana	863·3	871·8	866·7
V.	Karimganj	772·4	756·2	761·5
VI.	Pausra	643·5	662·7	631·2
VII.	Marh Bhangwan	282·9	275·7	277·1
VIII.	Chicharianwali	334·4	326·6	329·3
IX.	Titabar	2748·7	2762·8	2756·2
X.	Kaliganj	1100·9	1112·3	1116·5
XI.	Dacca	1233·1	1221·2	1238·6
XII.	Yessgaon No. 1	425·6	431·3	423·2
XIII.	Yessgaon No. 2	404·2	411·8	421·2
XIV.	Kopergaon	575·8	569·1	588·2
XV.	Malad No. 1	711·5	706·8	728·7
XVI.	Malad No. 2	744·9	751·1	741·6
XVII.	Karjat	570·6	562·7	566·0
XVIII.	Hmawbi	766·8	742·3	755·1
XIX.	Mandalay	545·1	561·1	532·7
XX.	Anakapalli	866·2	851·6	881·8
XXI.	Coimbatore F Block	1460·2	1479·1	1488·7
XXII.	Coimbatore M Block	630·8	610·1	600·3
XXIII.	Tirur	2556·6	2536·8	2563·5
XXIV.	Sholavandan	522·0	539·5	509·9

Average diminution ($\bar{x}$) ÷ Standard Error = t. $t = 2$ is taken as significant.
Values of t: 0–15th day = 0·4; 15–30th day = 0·3.

The Effect of Water-logging on the Reaction.

The hydrogen-ion concentration was determined by the method of drop-ratios (8), using the filtrate obtained from the water-logged soil after shaking up. The results are plotted in Graph 2.

There was a distinct rise in the *p*H values of the liquid, notably during the early stages (7·5–8·2 in seven days in the Rothamsted soil and 7·4–7·8 in four days in the Indian soil). There seemed to be a slight decrease after that time, but the figures still remained higher than at the beginning. The effects were much more marked in the Rothamsted than in the Indian soil.

Gillespie (9) has already remarked that water-logging tends to make soils less intensely acid. It should be observed that the rise in the *p*H value was more or less simultaneous with the increase in the free and saline ammonia content of the soils. An examination of the data showed clearly that there was a distinct positive correlation between the *p*H values and the logarithms of the corresponding amounts of free and saline ammonia.

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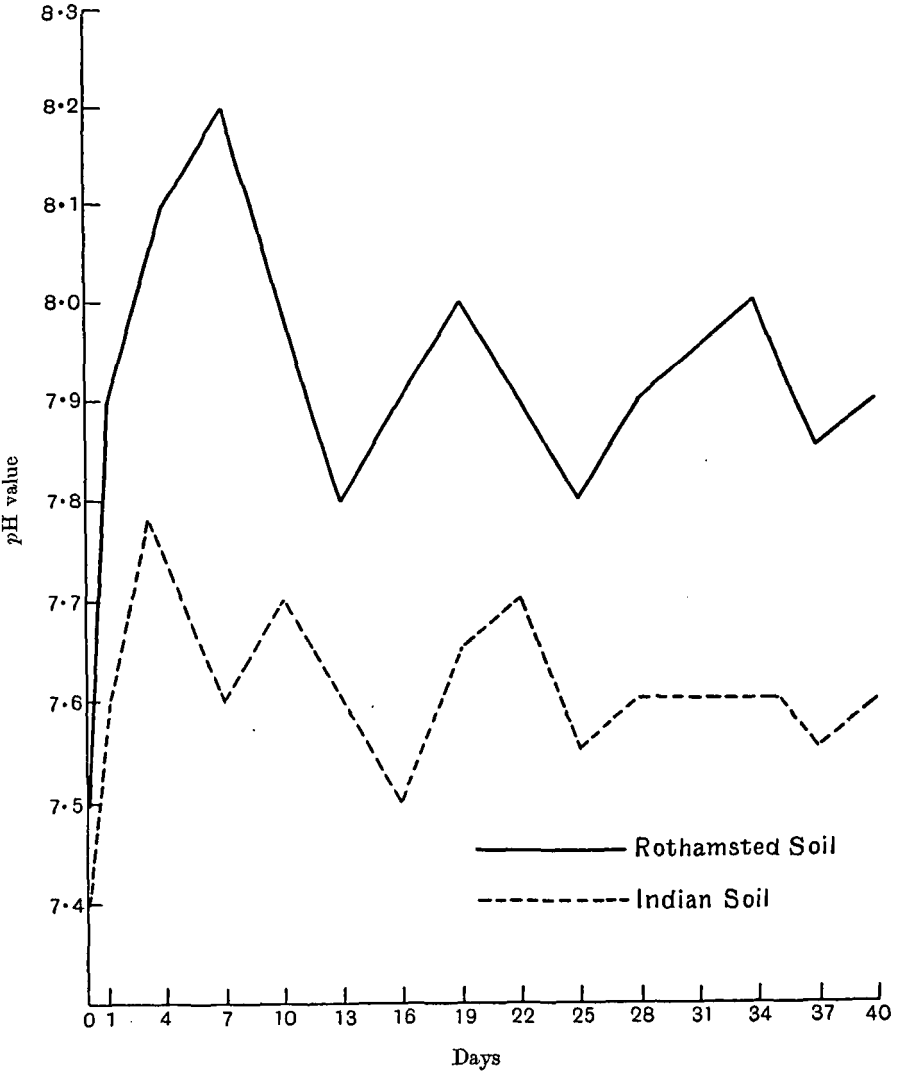
The Effect of Water-logging on the Gaseous Relations.

Oxygen absorption. Gillespie (9) has observed that there is a reduction potential set up in water-logged soils. In order to ascertain if that could



Graph 2. Changes in the Hydrogen-ion concentration.

be due to the formation of soluble reducing matter, a series of determinations of the oxygen absorbed by the soil extract at various times was carried out (16).

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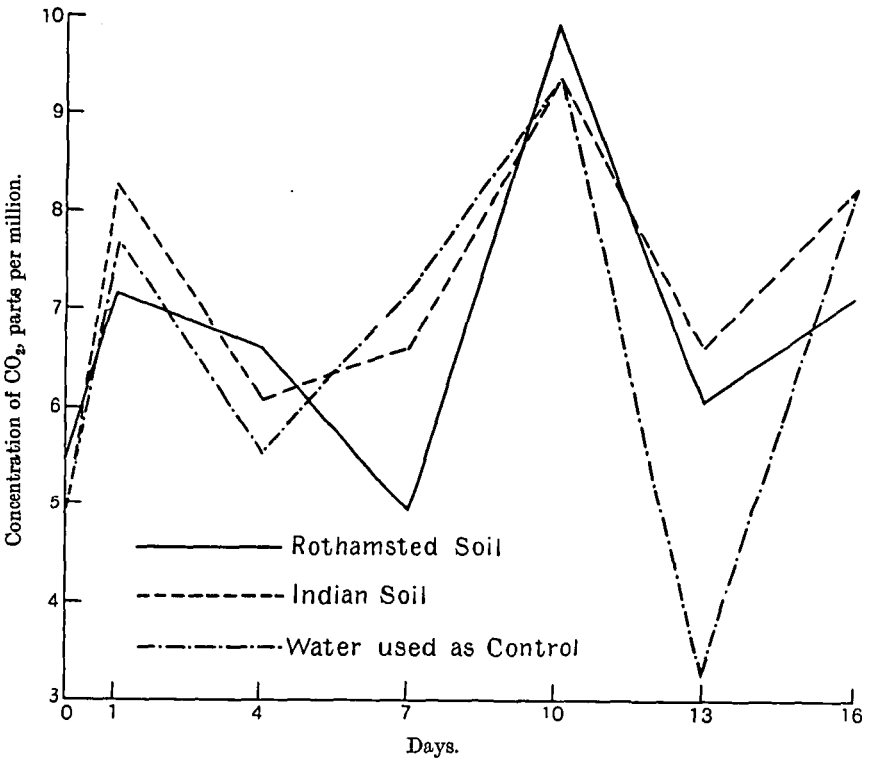
The results showed that whilst at the end of the first 24 hours there was a significant rise in oxygen absorption by the extract of one soil there was a similar fall in the absorption by the other. The figures for the later days showed marked rises and falls, but did not indicate any real difference in the absorption.

There is no evidence to suggest any appreciable variation in the absorption of oxygen by the extracts from the two water-logged soils.



Graph 3. Variations in the quantities of dissolved CO_2.

Production of carbon dioxide. Previous work(18) has shown that the production of ammonia from added protein matter in soils is generally accompanied by a corresponding evolution of carbon dioxide. In order to see if any similar correlation could be observed in the water-logged soils a series of determinations of the carbon dioxide formed at various times were carried out. The estimations were made by absorbing the gas in baryta contained in a flask connected to that containing the soil,

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and titrating the excess of the baryta against standard acid. The amount of carbon dioxide given off was found to be quite small.

A study of the quantities present in the dissolved state showed that though varying slightly they did not differ significantly from the quantities present in a sample of distilled water maintained under identical conditions as control. (Graph 3.)

It has already been pointed out that the increased production of ammonia could not be a purely chemical process and it was suggested that it might have been due to biological activity. But if the reaction had been brought about by any of the active ammonifying organisms (14 and 2) it must have been accompanied by the evolution of carbon dioxide. Since in the present case there is practically no carbon dioxide produced, the possible action of such organisms is precluded.

Dissolved Oxygen of the Surface Water.

Harrison and Iyer(11) have suggested that a probable source of oxygen to the swamp soils is that present dissolved in the surface water. In order to obtain an idea of the oxygen relations of the supernatant water a series of determinations of dissolved oxygen in it at different times was made. The estimations were made by Rideal and Stewart's modification of Winkler's method(22) with correction for the reducing matter of the soil.

A study of the results (Graph 4) shows that marked fluctuations in the content of dissolved oxygen occurred during the period of the experiments, but that these fluctuations were always in the same direction for the control and the water above the soil samples. The fluctuations were therefore due to variations in external conditions with time, and not to any soil factor.

In the case of the Rothamsted soil, which was kept at laboratory temperature, the individual values for the soil water and the control do not differ significantly, the differences being small and indiscriminately positive and negative. With the Indian soil, however, which was kept at a temperature of 35° C., the dissolved oxygen content of the soil water was less than that of the control at nearly every time of sampling. This appears to indicate that the latter soil was absorbing oxygen at a rate slightly faster than that at which it could be replenished from the atmosphere. If such absorption occurred with the Rothamsted soil it was so slow, at the lower temperature used, that its effect was masked by the supply of dissolved oxygen from the air.

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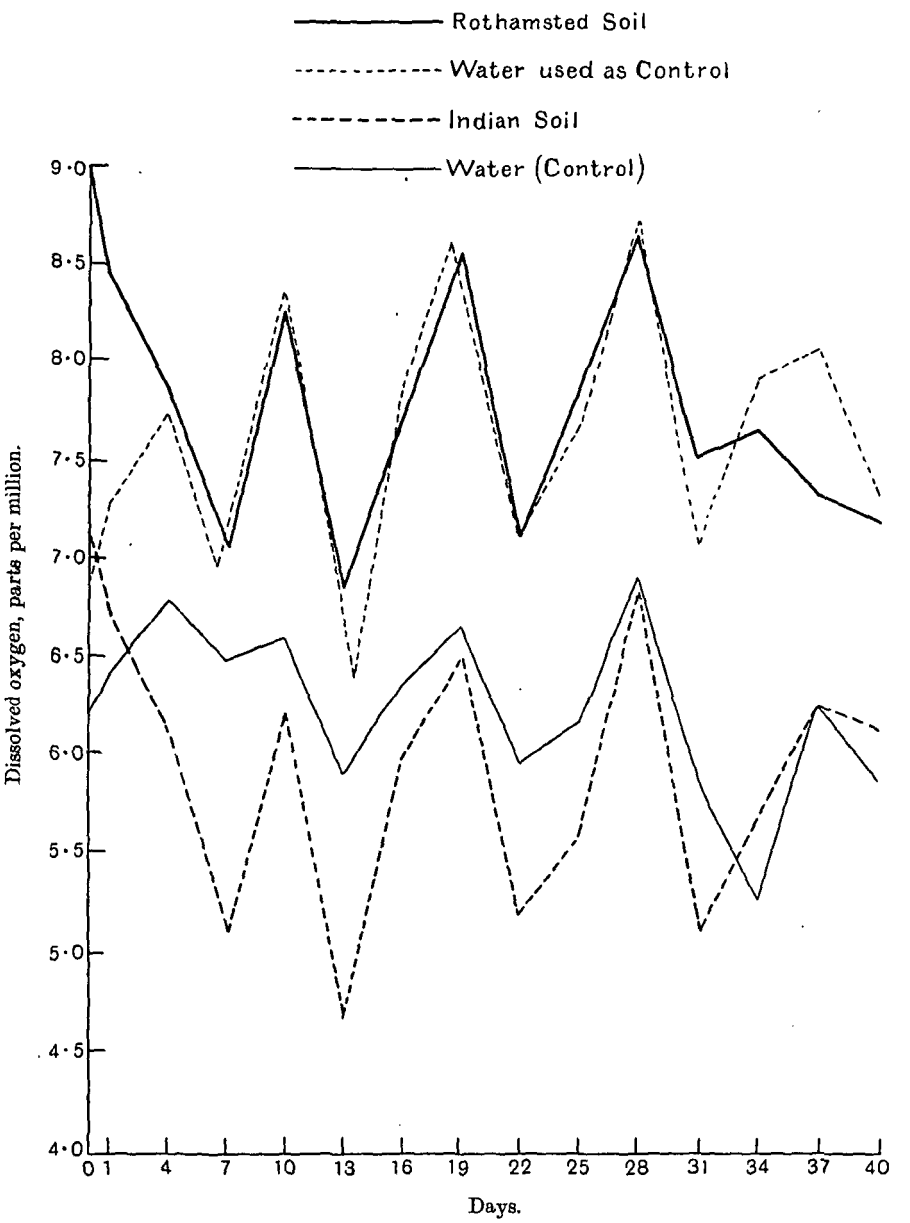

Graph 4. Variations in the Dissolved Oxygen of the surface water.

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The Diffusion of Oxygen from the Surface Water into the Soil.

In order to study quantitatively the diffusion of oxygen from the surface water into the soil 250 gm. lots of the soils were taken in Winchesters, saturated with water and partially deoxygenated by standing for a week connected with jars containing alkaline pyrogallol. The Winchesters were then filled with water which had been standing for some time under laboratory conditions and the oxygen content of which was steady, and tightly stoppered. After standing for 24 hours at laboratory temperature for each soil the oxygen content at successive one-inch depths was determined. The results were as follows:

Table IV.

Oxygen present originally in the water 84·7 P.P.M. Bar. P. 777·3 mm. Temp. 1·57° C.

Depth from the surface of the water (in inches)	Oxygen as parts per million			
	Rothamsted soil		Indian soil	
	Oxygen present	Oxygen lost	Oxygen present	Oxygen lost
1	8·35	0·12	8·41	0·06
2	8·04	0·43	8·28	0·19
3	7·63	0·84	7·78	0·69
4	6·58	1·89	6·91	1·56
5	5·57	2·90	6·46	2·01
6	5·05	3·42	5·35	3·12
7	4·75	3·72	5·08	3·39
8	4·52	3·95	4·81	3·66
9	4·47	4·0	4·63	3·84

The results show that the oxygen present in the soil was used up; dissolved oxygen from the surface water diffused downward and took its place.

An equation expressing the oxygen concentration-gradient in the water above a soil is

$$\log_e Y = K + pd + qd^2 + rd^3,$$

where Y is the decrement of oxygen content at a depth d below the surface, K is a constant for the soil, and p, q and r are constants independent of the soil, and determined by the rate of diffusion of oxygen through the liquid. In Graph 5 the logarithms of the decrements of oxygen content are plotted against the corresponding depths (Table IV). The curves obtained are fairly uniform and correspond closely to the equations:

$\text{Log}_e Y = 1{\cdot}264 + 0{\cdot}6x - 0{\cdot}09x^2 + 0{\cdot}0065x^3$ (for Rothamsted soil),

$\text{Log}_e Y = 0{\cdot}912 + 0{\cdot}6x - 0{\cdot}09x^2 + 0{\cdot}0065x^3$ (for Indian soil).

The actual quantities of oxygen present at any time at any given depth

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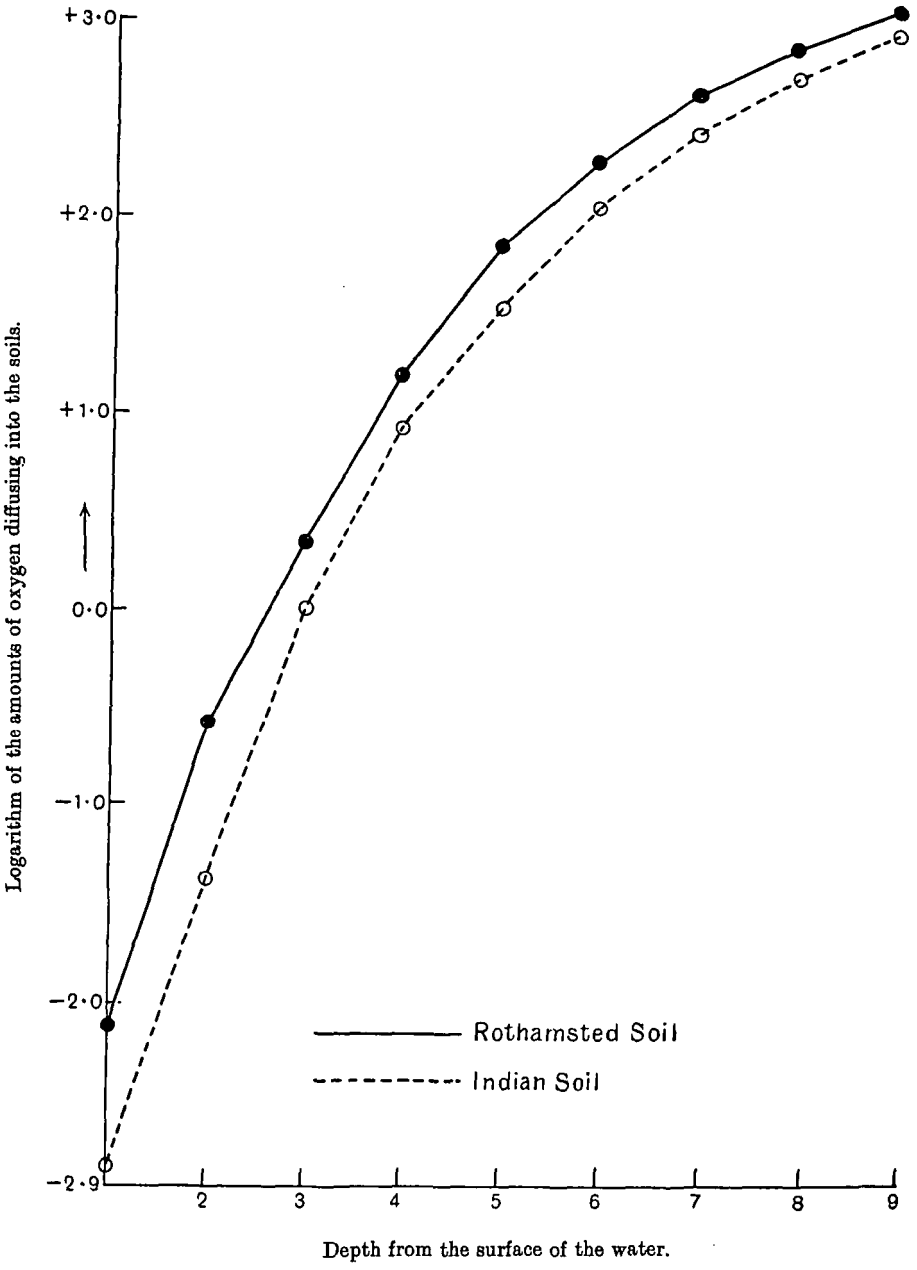

Graph 5. The diffusion of oxygen from the surface water into the soil.

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are determined by the rate of absorption of oxygen by the soils. The values of K for the two soils (1·264 for the Rothamsted soil and 0·912 for the Indian soil) show that the rate of absorption is greater for the former soil, when the temperature is the same for both.

The absorption of oxygen by these soils would appear to be non-biological in character, since it has been shown (p. 438) that there is no corresponding production of carbon dioxide.

The Effect of Water-logging on the Bacterial Flora.

Bacterial counts were made by plating on Thornton's Standardised Agar (25). The results are plotted in Graph 6.

There was a perceptible fall (37 to 26 M.P.G. in the Rothamsted soil and 25 to 16 M.P.G. in the other) in the numbers present at the end of the first twenty-four hours. Later the counts show a succession of rises and falls, many of which are significant. This observation is very similar to the one made during short period counts on arable soils (2). It is possible that in both cases the variations are caused by the presence of protozoa which are known to be particularly numerous in wet soils (3).

Giltay's Agar. In view of the fact that water-logging has frequently been associated with denitrification, platings were made under aerobic and anaerobic conditions on Giltay's Agar, which is a favourable medium for the isolation and development of denitrifying organisms (10).

The numbers of bacteria observed in the aerobic platings on this medium were much less than on Thornton's Agar and were highly inconsistent, the standard error being $\pm$ 10·9 per cent. (Graph 7). Some of the plates contained no colonies at all while many others were completely overgrown with fungi.

As was observed in the previous series the numbers show periodic rises and falls but there is no evidence that the general level rose or fell appreciably as a result of the water-logging.

In order to study the denitrifying properties of the organisms the more characteristic colonies appearing on the various plates were first developed in soil suspensions containing glucose (0·5 per cent.) and potassium nitrate (0·05 per cent.), then transferred into 100 gm. lots of the soils and incubated for three days at 35° and 20° C. Except for some slight reduction to nitrites no changes in the nitrate content could be observed at all.

The inconsistency of the counts combined with the tendency for increased development of fungi show that Giltay's Agar is not a suitable medium. Perhaps it is due to the fact that the concentrations of the

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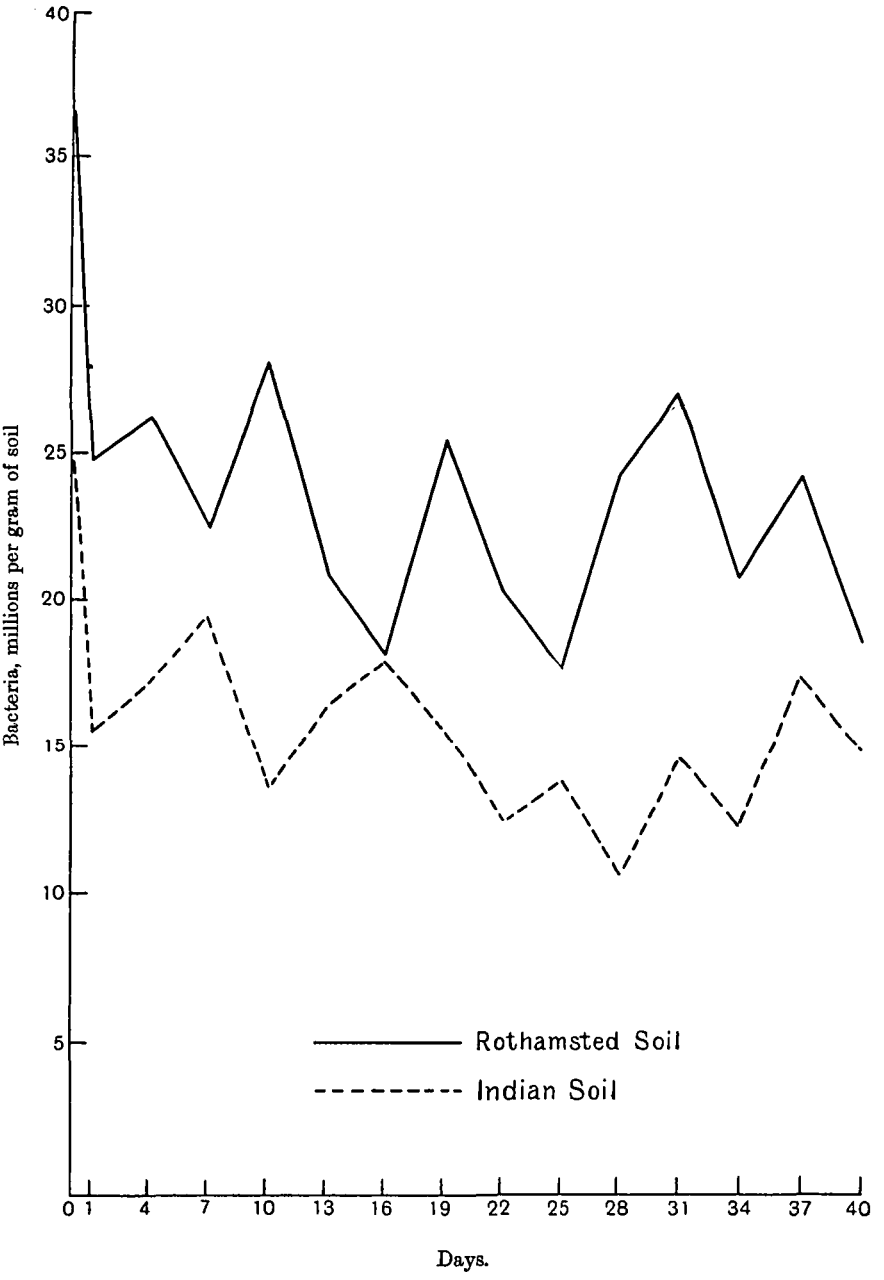

Graph 6. Variations in the bacterial counts, Thornton's Agar.

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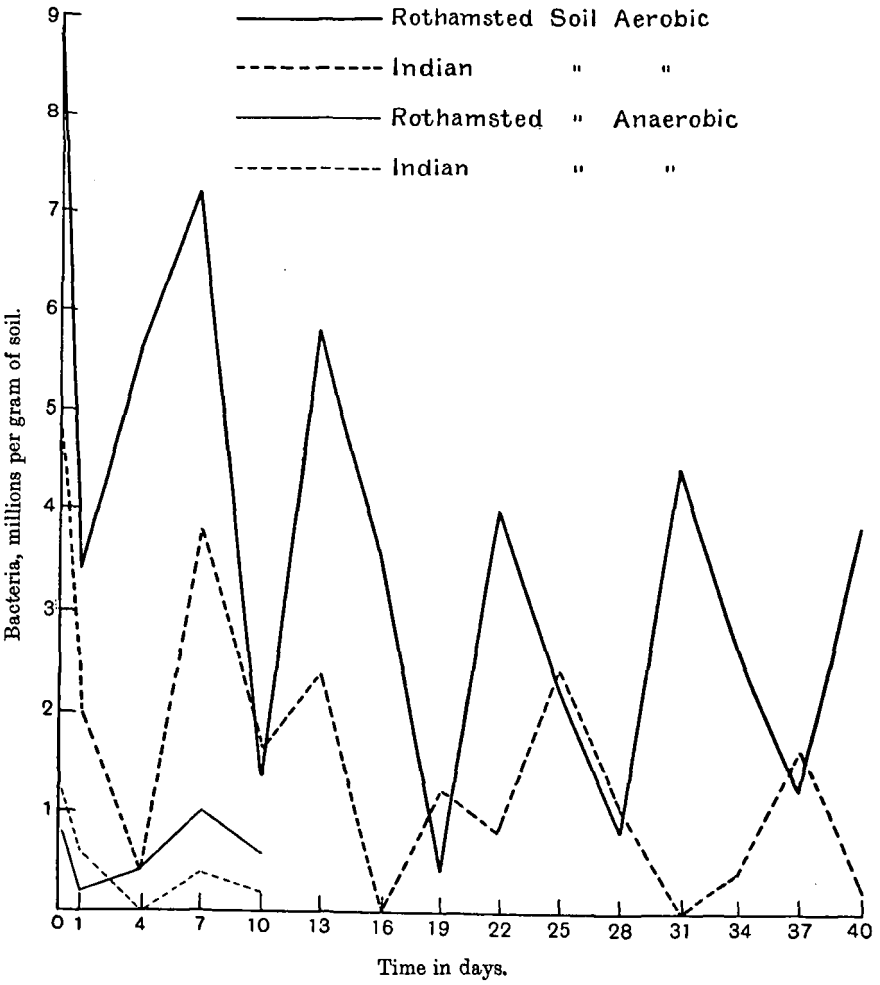
sugar and citric acid are too high as compared with the conditions normally prevailing in the soil.

The anaerobic platings were incubated in desiccators containing alkaline pyrogallol.



Graph 7. Bacterial counts on Giltay's Agar.

The numbers of colonies appearing after 10 days of incubation were very few, corresponding on the average to only one million per gram. (Graph 7.) None of them showed any nitrate-reducing power.

Soil extract-gelatine. Counts on soil extract-gelatine were obtained by plating on a medium composed of soil extract (sp. gr. 1·02) and gelatine (gold label, 18 per cent.) and incubating for five days at 20° C.

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under aerobic and anaerobic conditions. The gelatine-liquefying colonies were also separately counted.

Under aerobic conditions there was an appreciable fall during the first four days in the total counts of the Rothamsted soil (20–13), whereas with the Indian soil the fall was not so marked. (Graph 8.) As observed on the other media the numbers then varied periodically. They remained however significantly lower than at the outset, so that there is evidence for a slight diminution as a result of the water-logging. There were very few colonies of fungi on the plates.

The counts of the gelatine-liquefiers also varied from time to time but they do not suggest any real variation. They kept more or less constant while the total counts decreased.

Very few colonies (about 1 M.P.G.) came out on the anaerobic plates. The numbers showed a slight decrease at the end of twenty-four hours and the counts during the later days were somewhat lower than at the beginning. The gelatine-liquefiers formed a large proportion of the total number and as observed under aerobic conditions their numbers showed no real change as a result of the water-logging.

The Nature of the Agency responsible for Ammonification in Water-logged Soils.

Conn and Bright (2) have shown that when ammonification was brought about by biological activity there was a distinct increase in the numbers of certain non-spore-forming organisms which are active gelatine-liquefiers. If the increased ammonia production observed under the water-logged conditions had been brought about by the agency of such organisms we should expect to find an increase in the numbers of the gelatine-liquefiers. However no such increase took place, thus supporting the view that the reaction is probably not brought about by the activity of living organisms. The lack of appreciable carbon dioxide production also supports this view.

Russell and Hutchinson (23) have already noted that some ammonification takes place even where biological action is impossible and have suggested that it is probably brought about by enzyme action. A similar observation has been made by J. G. Lipman (13) who found that the quantity of ammonia produced from peptone was independent of the concentration of the latter. It is possible that in the present case we are dealing with a type of enzyme action.

The chemical mechanism of the reaction is apparently not one of oxidation. It is probably one of hydrolysis or reduction (1).

This question forms the subject of the next paper in this series.

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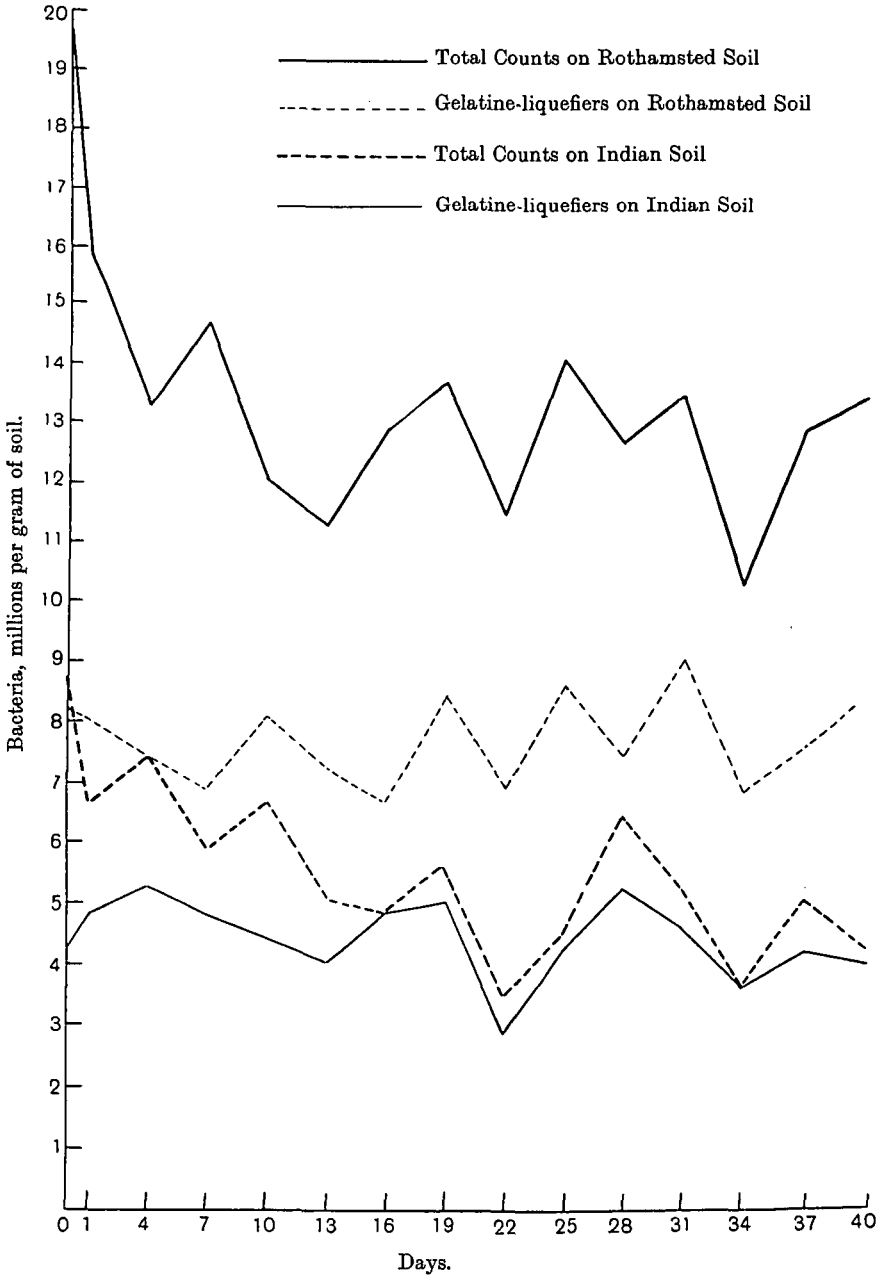

Graph 8. Bacterial counts on Soil Extract-Gelatine (Aerobic).

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SUMMARY AND CONCLUSIONS.

Rothamsted and Indian soils were water-logged in the absence of freshly decomposing organic matter.

Nitrogen changes: Water-logging resulted in:

(1) A distinct increase in the free and saline ammonia content.

(2) A significant though only slight diminution in the nitric nitrogen.

(3) No marked loss of ammonia by volatilisation or otherwise in the gaseous form: nor considerable variation in the nitrites: nor any observable diminution in the total nitrogen.

Reaction: Water-logging resulted in an increase in alkalinity; the increase in *p*H value was closely correlated with the corresponding increase in ammonia.

Gaseous relations: Water-logging resulted in:

(1) No release of any soluble reducing matter capable of absorbing dissolved oxygen.

(2) No appreciable carbon dioxide production.

(3) An absorption of dissolved oxygen from the surface water. An equation has been worked out expressing the concentration gradient of dissolved oxygen with depth.

Bacterial numbers:

From bacterial counts on water-logged soils it was found that:

(1) There was significant though slight decrease in bacterial numbers on Thornton's Agar.

(2) Very few colonies were obtained by plating aerobically (and fewer still anaerobically) on Giltay's Agar. None of the organisms appearing on the plates brought about any nitrate reduction in soils.

(3) The total counts on gelatine plates also showed some decrease. The numbers of gelatine-liquefiers on the other hand did not vary. There was no evidence to suggest that the increased production of ammonia was due to the activity of the gelatine-liquefiers.

Agency responsible for ammonia formation:

The results indicate that the formation of ammonia in water-logged soils is not due to biological action. It is suggested that the action is due to an enzyme.

In conclusion it is the pleasant duty of the author to acknowledge his indebtedness to Messrs H. J. Page and H. G. Thornton for their kind guidance and suggestive criticism.

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